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BIOCHEMICAL ANALYSIS OF IMPORTANT MEDICINALLY VALUABLE PLANTS BELONGING TO FAMILY *LILIACAE*

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ABSTRACT
In the present study chlorophyll, protein and ascorbic acid of selected medicinal plants of family Liliacae have been recorded. Plants provide biologically active metabolites and lead structures whose activities can be enhanced by manipulation through combinations with chemicals and synthetic chemistry. These metabolites can be exploited in the field of new drugs research and for the development of modified derivatives with enhanced activity and reduced toxicity. A total of five plant species were selected namely *Aloe vera*, *Allium sativum*, *Allium cepa*, *Asphodelus aestivus* and *Asparagus aphyllus* were used for analysis. The highest amount of total chlorophyll was found in *Aloe Vera* (4.38mg/g). The protein and ascorbic acid were maximum recorded in *Allium sativum* i.e 29.67 mg/100g and 5.72mg/100g.d.w respectively. These primary metabolites could be further used for biosynthesis of secondary metabolites or bioactive compounds. Results obtained show the great interest in plant pharmaceuticals.

KEYWORDS: *Liliacae*, Protein, Chlorophyll, Ascorbic acid, Medicinal plants.

INTRODUCTION
Medicinal plants belong to the oldest known health care products that have been used by mankind all over the world in the form of folklore medicines or traditional medicines or ethnic medicines. Even today, plants are the most exclusive sources of drugs for the majority of world's population and plant products constitute about sixty percent of prescribed medicine (Farnsworth and Bingel, 1997).

Plants are known to have a treasure ground of many kinds of biochemicals which have proved to be a boon for the whole of our mankind. These biochemicals are also referred to as phytochemicals (from the Greek word phyto, meaning plant), which are biologically active, naturally occurring chemical compounds found in plants (Verpoorte, 2000). These phytochemicals obtained as a result of various metabolic processes, occurring at different cellular levels involving many kinds and types of organic compounds, which further result in the production of various metabolites. These metabolites are grouped into primary and secondary metabolites. The metabolic pathways that are essential for sustaining life and organization of plant body are synthesis of primary metabolites *viz.*, protein, proline, carbohydrate, aminoacid, phenol, ascorbic acid, chlorophyll *etc*. These play recognized role in photosynthesis, respiration, transpiration, nutrient assimilation *etc*. The secondary metabolites are having a restricted distribution in plant kingdom. Nevertheless, secondary metabolites are found specific to a particular plant species or a taxonomically related group of species; whereas the basic primary metabolites are found throughout the plant kingdom (Taiz and Zeiger, 1996).

The Liliaceae or lily family is composed of large number of plant with medicinal virtues. Most of these are herbs and rarely shrubs. Examples from this plant family include Asphodel (*Asphodelus aestivus*), Wild asparagus (*Asparagus aphyllus*), Aloe (*Aloe vera*), Garlic (*Allium sativum*), Garden onion (*Allium cepa*) etc.Wild asparagus (*Asparagus aphyllus*), its underground rooting system was used as a diuretic, antispasmidic and sedative. Aloe (*Aloe vera*), two extracts are obtained from Aloe, a yellowish-green juice and a gel from the fleshy leaves. When the leaves are cut, the exuding juice contains constituents with a laxative effect. The skin of the leaves can be removed to obtain the gel. The gel is widely used in several preparations such as skin and hair products. Garlic (*Allium sativum*) is a rather cultivated plant; the bulb of the plant is used medicinally, in the fresh, dried or processed state. Garden onion (*Allium cepa*) is closely related to the onion. The fresh bulb is used for medicinal purposes. It has strong antiseptic properties related to the organic sulphur compounds present in the bulb. Asphodel (*Asphodelus*

aestivus), It usually bears white large flowers. In folk medicine, they have been used to reduce pigmentation of the skin and to stop wound bleeding.

MATERIALS AND METHODS

Collection of plants: Healthy plants of *Aloe vera*, *Allium sativum, Allium cepa, Asphodelus aestivus* and *Asparagus aphyllus* were collected from Chittorgarh, Rajasthan and adjoining areas. Plant was authenticated by the Herbarium, Department of Botany, MM Modi College, Modinagar, Uttar Pradesh, India. All the data were analyzed statistical methods using one way Analysis of Variance (ANNOVA).

Estimation of Chlorophyll: For estimation of chlorophyll the method of Hiscox and Israelstam (1979) was followed. 50 mg of leaf materials from fully emerged leaf was weighed and put in the 10ml of Dimethyl sulphoxide (DMSO). The test tubes were kept in the oven at 65^0C for 4hto facilitate extraction of chlorophyll into the solution. The absorbance (A) values was then recorded at 645 and 663 nm in a spectrophotometer.The amount of total chlorophyll was calculated using following formula....

Total Chlorophyll $= 22.2\ (A_{645}) + 8.02\ (A_{663})$

Estimation of Protein: Soluble protein in the leaves was determined by the method given by Bradford (1976).100 mg of Coomassie Brilliant blue dye (G-250) was dissolved in 50 ml of 95% ethanol, 100ml concentration of orthophoric acid was added to it. Final volume was made to 200 ml with distilled water. The solution was kept in a amber colour bottle at 4^0c. 500 mg fresh leaves was ground using pestle and mortar with 3 ml of cold extraction buffer (60mM Tris-HCl), pH 6.9. The extract was centrifuged at 15000 rpm for 15 min. the supernatant was made to 5 ml with extraction buffer. It was used as stock crude protein. The dye concentration was diluted five times with distilled water (1 dye conc.: 4 distilled water). Filter with Whatman No. 1 paper if ppt. occurs. To 5ml of diluted dye 30 ml of leaf crude protein and 80 ml water was added. The contents were vortexed and absorbance was measured at 595 nm after 10 min.

Estimation of Ascorbic acid: Ascorbic acid is an important chemical antioxidant, which is responsible for the non-enzymatic scavenging of superoxide radical and hydrogen peroxide, regeneration of tocopherol in chloroplast and in enzymatic scavenging of H_2O_2 in association with ascorbate peroxidase. Its estimation is based on the formation of pink coloured complex due to the reduction of dinitrophenylhydrazine by ascorbic acid to phenyl hydrazone in acidic medium (Mukherjee and Choudhuri 1983). Fresh leaf sample (0.5 g) or preserved in liquid nitrogen is extracted with 10 ml of 6% trichloroacetic acid. Homogenate is centrifuged at 5000 g at 4 ^{0}C temperature in a refrigerated centrifuge. Supernatant is used for estimation of ascorbic acid. Four ml of the extract is mixed with 2 ml of 2% dinitrophenylhydrazine (in acidic medium) followed by the addition of 1 drop of 10% thiourea (in 70% ethanol). The mixture is heated for 15 min in a boiling water bath (100^oC), and after cooling to room temperature, 5 ml of 80% (v/v) H_2SO_4 added to the mixture at 0^oC (in an ice bath). The absorbance is recorded at 530 nm.

RESULTS AND DISCUSSION

In the present investigation, five medicinal plants *Aloe vera*, *Allium sativum, Allium cepa, Asphodelus aestivus* and *Asparagus aphyllus* were evaluated quantitatively for the analysis of total soluble protein, chlorophyll and ascorbic acid. The results are present in Table-1. Total levels of Chlorophyll were found to be maximum in leaf of *Aloe vera* i.e. 6.53 mg/g and minimum in *Asparagus aphyllus* i.e. 4.38 mg/g (Fig 1). Total levels of protein were found to be maximum in *Allium sativum* i.e. 29.67 mg/g. and minimum amount in leaf of *Asphodelus aestivus* i.e.15.55 mg/g (Fig 2). The levels of ascorbic acid were also maximum found *Allium sativum* i.e. 5.72 mg/g and minimum in *Asparagus aphyllus* i.e. 1.10 mg/g (Fig 3).

Table 1: Biochemical analysis of selected medicinal plants leaves belonging to family *Liliacae.*

S. No.	Plants	Chlorophyll (mg/g)		Protein (mg/g)		Ascorbic acid (mg/g)	
		Mean	S.E.	Mean	S.E.	Mean	S.E.
1	*Aloe vera*	6.53	± 0.63	21.74	± 0.54	3.56	± 0.58
2	*Allium sativum*	4.34	± 0.57	29.67	± 0.92	5.72	± 0.74
3	*Allium cepa*	5.70	± 0.82	22.39	± 0.57	1.98	± 0.23
4	*Asphodelus aestivus*	4.75	± 0.23	15.55	± 0.40	3.63	± 0.35
5	*Asparagus aphyllus*	4.38	± 0.25	15.63	± 0.61	1.10	± 0.10
	C.D.	1.13		9.46		0.98	
	SE(m)	0.34		2.86		0.30	
	SE(d)	0.48		4.04		0.42	
	C.V.	11.50		23.57		18.24	

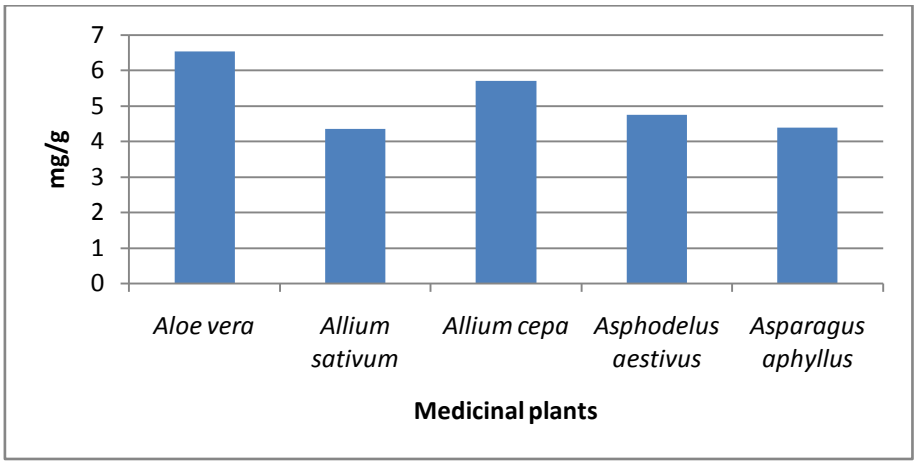

Figure 1- Estimation of total chlorophyll.

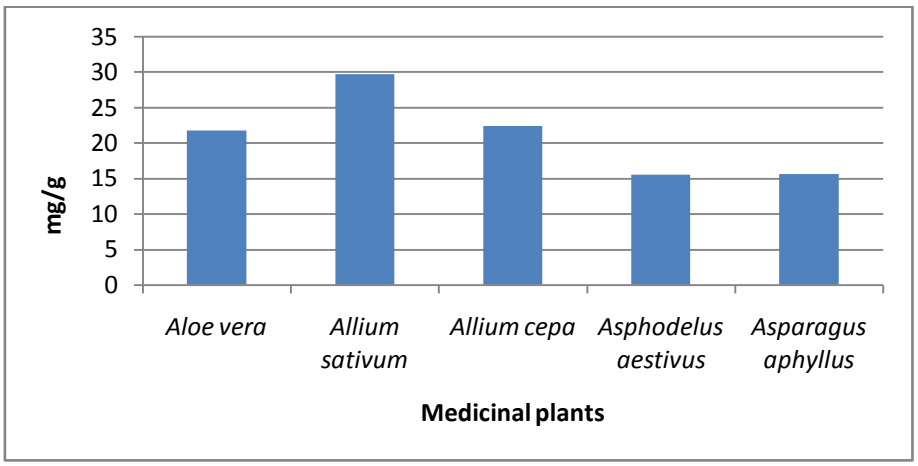

Figure 2- Estimation of Protein.

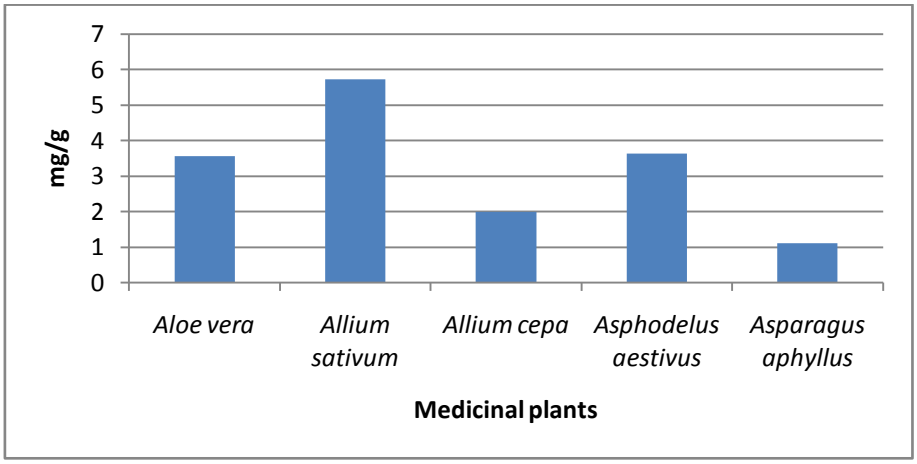

Figure 3- Estimation of Ascorbic acid.

The traditional medicine, which has chemical compounds derived from medicinal plants. These compounds are classified into primary and secondary metabolites (Vinoth et. al., 2011). Primary metabolites are of prime importance and essentially required for growth of plants. Many primary metabolites lie in their impact as precursors or pharmacologically active metabolites in of pharmaceutical compounds such as antipsychotic drugs. The present work is to analyze three basic primary metabolites (protein, chlorophyll and ascorbic acid) of selected plants. Green plants have different characters because of the presence of various pigments like chlorophyll, carotenoid, other pigments and water content which together constitute the spectral characters of a plant body (Philip and Shirly, 1978; Jan-Chang Chen and Chaur-Tzuhn Chen, 2006). However the chlorophyll content has medicinal qualities. The chlorophyll is also plays important role in plant physiology and it can be act as nutrition in decline blood sugar conditions, detoxification, digestion, excretion and decreasing allergens (Srichaikul *et al*., 2011, Singh et al., 2011). Chlorophyll is the most indispensable class of primary compounds as they are the only substances that capture sunlight and make it available to plant system for its cultivation on photosynthesis (Murray et. al., 1986).

Proteins are biochemical compounds consisting of one or more polypeptides typically folded into a globular or fibrous form, facilitating a biological function. Proteins are the chief actors within the cell, said to be carrying out the duties specified by the information encoded in genes (Lodish et. al., 2004). Proteins are the primary components of living things. The presence of higher protein level in the plant points towards their possible increase food value or that a protein base bioactive compound could also be isolated in future (Thomsen et. al., 1991).

Ascorbic acid is an important regulator of oxidation and plays a significant role in germination (Key 1962). The role of ascorbic acid in plant growth and metabolism has been studied by various workers (Isherwood and Mapson 1962). Ascorbic acid (vitamin C) is a familiar molecule because of its dietary significance, it is not only an important antioxidant, it also appears to link flowering time, developmental senescence, programmed cell death and responses to pathogens through a complex signal transduction network (Nicholas, 1996 and Mapson, 1958).

CONCLUSION

Economic use of plants depends partially on the quantitative and qualitative aspects of these organic reserves, especially chlorophyll, proteins, and ascorbic acid. In the present investigation comparison of various primary metabolites from samples of Medicinal plants had been carried out. This study suggests that plant parts having rich primary metabolites can be used industrially as raw materials having commercial importance. Variation in biochemical parameters like chlorophyll, protein and ascorbic acid in the leaves were found to be pollution load dependent. These variations can be used as indicators of air pollution for early diagnosis of stress or as a marker for physiological damage to plants prior to the onset of visible injury symptoms. Just by analyzing these biochemical indicators air qualities can also be assessed.

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